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Effects of Glycine Betaine on Plant Growth and Performance of Alfalfa (Medicago Sativa L.) & Cowpea (Vigna Unguiculata L. Walp.) Within Water Deficit Conditions

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EFFECTS OF GLYCINE BETAINE ON PLANT GROWTH AND PERFORMANCE OF ALFALFA (*MEDICAGO SATIVA* L.) & COWPEA (*VIGNA UNGUICULATA* L. WALP.) WITHIN WATER DEFICIT CONDITIONS

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This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Horticulture

Under the Supervision of Dr. Mohammed Abdul Mohsen Salem Al-Yafei

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Declaration of Original Work

I, Hanan Kamal Khadouri, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled **"**Effects of Glycine Betaine on plant growth and performance of Alfalfa (*Medicago sativa* L.) & Cowpea (*Vigna unguiculata* L. Walp.) within water deficit conditions**"**, hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Dr. Mohammed Abdul Mohsen Alyafei, in the College of Food and Agriculture at UAEU. This work has not previously been presented or published, or formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

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Abstract

Managing water consumption of the crops is one of the strategies which have been adopted locally and worldwide in current trends of sustainable agriculture, in addition to the low level of water resources in UAE because of the rare precipitation, no fresh free flowing water; no rivers, lakes or streams. The present study was objective to evaluate the effects of organic exogenous Glycine Betaine on two forage crops Alfa alfa (*Medicago sativa* L.) & Cowpea (*Vigna unguiculata* L. Walp.) within different levels of drought stress. A pot experiment was designed on completely randomized block design (CRBD) with three replicates in each treatment in each plant. Seeds were germinated and the plants watered as required. Exogenous Glycine Betaine was applied as foliar spray in three concentrations (0,100 and 200 ppm) for five times with five days intervals. Water deficit stress start within the second Glycine Betaine treatment in three levels (24h: 100% well-watered recommended), (48h: 60% water deficit) and (72h: 40% water deficit) depending on the required quantity. Growth characteristics, pigment concentrations, biochemical content and Mineral nutrients levels were measured in response to the treatment variables. Results show Glycine Betaine has significant increment in Growth parameters, biochemical contents and Mineral nutrients concentrations. As a conclusion the results suggest that exogenous applications of Glycine Betaine were improve the drought tolerance in Cowpea and has enhanced the Alfa alfa performance under water deficit stress in both concentrations 100 & 200 ppm under drought stress of 60% of irrigations water . In due of comparison of Cowpea and Alfa alfa in their response to Glycine Betaine under the water deficit conditions, it’s found from this study that Glycine Betaine has better effect on the Cowpea under drought stress than Alfa alfa.

Keywords: Drought stress, exogenous osmo-regulator Glycine Betaine (GB), compatible solutes, Alfa alfa & Cowpea, morphology, pigments, biochemical and mineral nutrients.

Title and Abstract (in Arabic)

دراسة تأثير الجلايسين بيتاين على نمو وأداء النباتين الجت الحجازي واللوبياء العلفية ضمن ظروف عجز المياه

الملخص

تعتبر أدارة أستهلاك المياه للمحاصيل الزراعية أحدى الستراتيجيات التي تم تبنيها مؤخراً محلياً وعالمياً ضمن التوجهات الحالية نحو الزراعة المستدامة. بالأضافة الى انخفاض معدلات مصادر المياه في دولة الأمارات العربية المتحدة بسبب ندرة الأمطار وقلة توافر المياه العذبة لعدم توفر الأنهار والبحيرات. فقد هدفت الدراسة الحالية الى تقييم تأثير أضافة المادة العضوية الجلايسين بيتاين خارجياً على أثنان من نباتات المحاصيل العلفية، الجت الحجازي (*Medicago sativa* L.) واللوبياء العلفية (*Vigna unguiculata* L. Walp.)، ضمن مستويات مختلفة في إجهاد الجفاف.

تمت التجربة ضمن الأصص الزراعية على أساس تصميم القطاعات العشوائية المتكاملة ضمن ثلاثة مكررات في كل معاملة. كانت زراعة البذور وريها طبقاً للمتطلبات الزراعية لكل نبات. أجريت المعاملات الخارجية لمادة الجلايسين بيتاين من خلال رش النباتات ورقياً وعلى ثلاثة تراكيز (0 ، 100 و 200 جزء بالمليون) وعلى خمسة رشات بفاصل خمسة أيام بين كل رشة وأخرى. أبتداءت معاملات إجهاد عجز المياه على النباتات بعد أجراء الرشة الثانية للجلايسين بيتاين، وكانت بثلاثة مستويات ري (كل 24 ساعة: 100% - الري الموصى به)، (كل 48 ساعة: 60% من مياه الري) و(كل 72 ساعة: 40% من مياه الري) على أساس الكمية الموصى بها للنمو للنبات.

شملت الدراسة معايير متعددة منها، معدلات النمو، المحتوى الكمي لصبغيات البناء الضوئي، المكونات الكيميائية الحيوية وتراكيز المعادن الغذائية، نسبة الى أستجابتها الى متغيرات التجربة: إجهاد عجز المياه والجلايسين بيتاين.

أظهرت النتائج تأثير الجلايسين بيتاين في أعطاء زيادة ملحوظة في معدلات النمو ومحتوى المكونات الكيميائية الحيوية والمعادن الغذائية ضمن ظروف الجفاف. وكنتيجة نهائية يقترح من النتائج الحاصل عليها، بأن أضافة الجلايسين بيتاين يحسن من تحمل نبات اللوبياء العلفية لظروف الجفاف ويعزز أداء نبات الجت الحجازي تحت ظروف نقص المياه في كلا التركيزين (100 و 200 جزء بالمليون) وتحت ظروف جفاف 60% من مياه الري.

وللمقارنة بين أستجابة اللوبياء العلفية والجت الحجازي الى الجلايسين بيتاين ضمن ظروف عجز المياه وجد من خلال هذه الدراسة بأن تأثير الجلايسين بيتاين كان أفضل على اللوبياء العلفية ضمن ظروف الجفاف عما هو عليه في نبات الجت الحجازي.

مفاهيم البحث الرئيسية: إجهاد الجفاف، جلايسين بيتاين العضوي الخارجي، المحاليل المتوافقة، الجت الحجازي، اللوبياء العلفية، مظاهر النمو، الصبغيات البناء الضوئي، المكونات الكيميائية الحيوية، العناصر الغذائية.

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Dedication

To my beloved father and mother

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Chapter 1: Introduction

1.1 Overview

As UAE situated in a region of Arid to Semi-arid environment, it forced to face many challenges in agriculture sector; like drought and water deficiency which considered the most important problem at the region. Securing a safe and sustainable water supply, both now and in the future, presents a profound challenge, which, if left unaddressed, threatens the economic, social and environmental well-being. Executive Director of EAD's Integrated Environment Policy & Planning Sector, Dr. Mohamed Yousef Al Madfaei said "The scale of the water challenge facing Abu Dhabi cannot be overstated. Only by embracing our collective responsibilities and working together on sustainable water solutions can we hope to mitigate the burgeoning economic, social and environmental impacts. We have reached the tipping point, and our current rate of abstraction and use of groundwater is unsustainable. (Environment Agency – Abu Dhabi (EAD) 2012).

1.2 Water Deficit Stress:

Permanent or temporary water deficit stress limits the growth and distribution of natural and artificial vegetation and the performance of cultivated plants (crops) more than any other environmental factor. Productive and sustainable agriculture necessitates growing plants (crops) in arid and semiarid regions with less input of precious resources such as fresh water.

For a better understanding and rapid improvement of soil-water stress tolerance in these regions, especially in the water-wind eroded crossing region, it is very important to link physiological and biochemical studies to molecular work in genetically tractable model plants and important native plants, and further extending them to practical ecological restoration and efficient crop production.

Although basic studies and practices aimed at improving soil water stress resistance and plant water use efficiency have been carried out for many years, the mechanisms involved at different scales are still not clear. Further understanding and manipulating soil-plant water relationships and soil-water stress tolerance at the scales of ecology, physiology and molecular biology can significantly improve plant productivity and environmental quality. (Shao et al., 2009)

Forage grass like alfalfa and cowpea are required in the area for important proposes like grazing and soil conserving but their growth is highly dependent on soil moisture, and therefore needs to adopt alternative ways to maintain the water consumption of the plants within conditions of low precipitation in the country.

1.3 Alfa alfa & Cowpea:

Alfalfa (*Medicago sativa* L.) is one of the main fodder crops grown in the UAE. This specie requires large quantities of water (up to 15,700 m^3 ha^{-1} yr^{-1}), often drawn from non-renewable groundwater sources. Large-scale cultivation of Alfa-alfa to meet the increased demand for forage has resulted in drastic reduction in groundwater levels and an increase in salinity due to intrusion of seawater, especially in the coastal areas. At the current rates of agricultural use, it is predicted that all of UAE's fresh and moderately brackish water resources will be exhausted in 20-40 years (EAD, 2009).

Cowpea (*Vigna unguiculata* (L.) Walp.] is an important food legume and a valuable component of the traditional cropping systems in semi-arid tropics covering Asia, Africa, Central and South America. Cowpea has many uses: the young leaves, green pods and green seeds are used as vegetables; and dry seeds are used in various food preparations (Singh et al., 2003).

Drought adaptation in cowpea has been related to the minimization of water losses by the control of stomatal aperture (de Carvalho et al., 1998). Stomatal regulation of photosynthesis during water shortage has been well documented (Chaves, 1991). At least under mild drought conditions, it has been shown that stomata play the dominant role in controlling the decline of net CO2 uptake, by leading to decreases in leaf internal CO2 concentrations (Cornic and Briantais, 1991; Cornic, 2000).

Cowpea is equally important as a nutritious fodder for livestock. The crude protein content in the grain and leaves ranges from 22 to 30% on a dry weight basis (in comparison, alfalfa has 18 to 20% protein) and from 13 to 17% in the haulms with a high digestibility and low fiber level (Tarawali et al., 1997).

1.4 Role of Organic Glycine betaine in plants as osmo-regulators (GB):

Glycine betaine (GB) is an organic compound that occurs in plants, it is an amphoteric quaternary amine, plays an important role as a compatible solute in plants under various types of environmental stress, such as high levels of salts and high or low temperature (Sakamoto A. and Murata N., 2002). Compatible solutes are low molecular weight, highly water-soluble compounds that are usually non-toxic at high cellular concentrations. Generally, they protect plants from stress through different courses, including contribution to cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity, and stabilization of enzymes/proteins (Yancey et al., 1982; Bohnert and Jensen, 1996; Giri, 2011).

Furthermore, because some of these solutes also protect cellular components from dehydration injury, they are commonly referred to as osmo-protectants. These solutes include proline, sucrose, polyols, trehalose and quaternary ammonium compounds (QACs) such as glycine betaine, alanine betaine, proline betaine, choline O-sulfate, hydroxyl proline betaine, and pipecolate betaine (Rhodes and Hanson, 1993).

These solutes are biologically inert and accumulate at high concentration in the cytoplasm without interfering with the overall cellular functions; hence they are called compatible solute (Brown, 1978; Smiatek et al., 2012). Further biological importance is given by the stabilization of proteins in presence of compatible solutes (Lamosa, et al., 2003; Foord, 1998), Most of the theories prefer an indirect mechanism in which the compatible solute does not directly interact with the macro molecule. An important theory in this context is the preferential exclusion model which states that the appearance of the co-solvent leads to thermodynamic interactions with the protein (Timasheff, 2002; Lee and Timasheff, 1981). Due to these interactions, the protein repels the co-solvent from its surface to the bulk region. Thus the concentration of the co-solvent is lower in close vicinity to the protein whereas its bulk value is increased. This can only be accomplished by the addition of excess water molecules to the proteins surface which results in a preferential hydration of the macromolecule. The excess water molecules finally conserve the native form such that unfolding becomes less favorable which is realized by an increase of the melting temperature (Yu, 2004; Yu, et al., 2007; Knapp et al., 1999; Yancey, et al., 1982).

GB is abundant mainly in chloroplast where it plays a vital role in adjustment and protection of thylakoid membrane, thereby maintaining photosynthetic efficiency (Robinson and Jones, 1986; Genard et al., 1991). Glycine betaine is an oxidation product of choline in a biosynthetic pathway leading to methionine; it can donate a methyl group to homocysteine to form methionine.

GB is synthesized in chloroplast from serine via ethanolamine, choline, and betaine aldehyde (Hanson and Scott, 1980; Rhodes and Hanson, 1993).

Choline is converted to betaine aldehyde, by choline mono-oxygenase (CMO), which is then converted to Glycine Betaine by betaine aldehyde dehydrogenase (BADH) (Ashraf and Foolad 2007), (Hanson and Scott, 1980; Rhodes and Hanson, 1993) (Fig. 1). This reaction is the only known biological methylation that does not involve S-adenosyl-methionine.

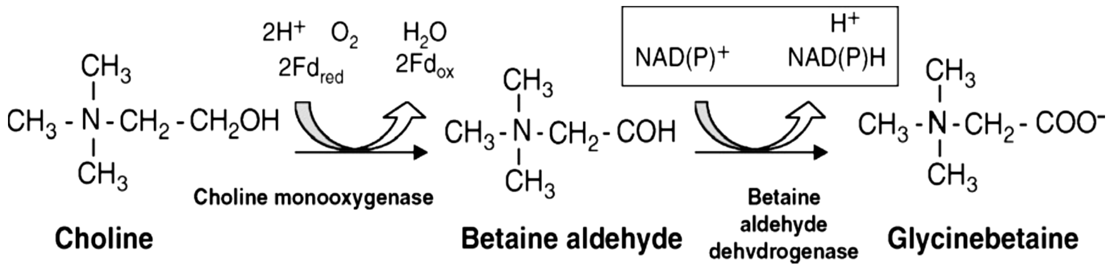

Figure 1**:** Glycine betaine synthesized path

Two complementary mechanisms are recognized for the protective effects of organic osmolytes in cells exposed to hypertonicity (Yancey et al., 1982). “Perturbing” Versus “Compatible” Solutes, although many biochemical functions require specific inorganic ions, increasing the concentrations of these ions above those typically found in cells perturbs protein function. In contrast, organic osmolytes have much less effect, i.e. are compatible. Hypertonicity, as results from high NaCl, causes osmotic flux of water out of cells, elevating the concentration of all cellular constituents, including inorganic salts (Burg et al., 2007). Although cells can rapidly restore their volume (“regulatory volume increase”) by influx of inorganic salts followed by osmotic uptake of water, the intracellular inorganic ion concentration remains high. Organic osmolytes accumulate later, associated with a decreasing concentration of intracellular inorganic salts. Thus, perturbing inorganic ions are replaced by compatible osmolytes (Burg and Ferraris, 2008).

1.5 Objectives of the present study are:

- Examine the effect of applying the exogenous organic osmolet Glycine Betaine on the growth and performance of two forage plants Alfalfa (*Medicago sativa* L.) & Cowpea (*Vigna unguiculata (L.) Wale.*), which have been exposure on irrigation regimes within the stress conditions of drought and high temperature in UAE environment.
- Determine the minimum level of irrigation can the plants adopted with support of GB and compared with the controls for each of alfalfa and cowpea that have not applied with GB.
- Comparison between these two forage plants in their responses to Glycine Betaine within irrigation regimes exposure.

Chapter 2: Literature Review

2.1 Effect of drought and water stress:

As global climate change continues, water shortage and drought are becoming an increasingly serious constraint, limiting crop production worldwide (Katerji et al., 2008), Recently the available amount of water in agriculture is declining worldwide because the rapid population growth and the greater incidence of drought caused by climate change and different human activities (World Bank, 2006).

Plant water stress can limit productivity and has an effect on plant physiology and canopy structure. Numerous researches have been carried out on the relationship between growth of alfalfa and water utilization (Ogata et al., 1960; James and Wright, 1988; Sheaffer, 1988; Smeal et al., 1991; Geng et al., 1995) and theorized on a positive linear relationship between yield and actual transpiration. Some researchers reported that water stress affected the transpiration ratio of alfalfa and its morphology (Brown and Tanner, 1983; Matthias and Smith; 1997).

The Crop Water Requirements (CWR) differs among cropping systems (Han and Qu, 1991; Liu and Han, 1987) and could be significantly impacted by climate change (Cao et al., 2008; Liu and Lin, 2004; Ma et al., 2006; Sun et al., 2013).

Inhibitory effect of water stress on root nodule activity in legumes (Engin and Sprent, 1973; Finn and Brun, 1980; Sprent, 1976), including alfalfa (Aguirreolea and Sanchez-Diax, 1989; Aparicio-Tejo et al., 1980). Deficit irrigation provides a means of reducing water consumption while minimizing adverse effects on yield (English and Nakamura, 1989; English and Raja, 1996; Mugabe and Nyakatawa, 2000; Bazza, 1999; Ghinassi and Trucchi, 2001; Kirda, 2002; Mao et al., 2003; Panda et al., 2003; Zhang et al., 2004).

2.2 Compatible solutes in plants:

Metabolites that might be expected to contribute to enhanced stress tolerance include soluble sugars, amino acids, organic acids, polyamines, and lipids (Guy, 1990). One important group of such metabolites includes the so-called ‘compatible solutes’, which are small organic metabolites that are very soluble in water and are non-toxic at high concentrations. One of the best studied compatible solutes is glycine betaine (N,N,N-tri-methyl-glycine, abbreviated as GB; (Chen and Murata, 2002; Chen and Murata, 2008).

Most organisms increase the cellular concentration of osmotically active compounds, termed compatible solutes, when in danger of becoming desiccated by either drought or external lowering of the osmotic pressure accompanying, for example, increases in soil salinity (Yancey et al., 1982; Le Rudulier et al., 1984; Mc Cue and Hanson, 1990; Delauney and Verma, 1993). The accumulating compounds are `compatible' with normal cellular metabolism at high concentrations (Brown and Simpson, 1972). Typically, compatible solutes are hydrophilic giving rise to the view that they could replace water at the surface of proteins, protein complexes, or membranes. Compatible solute is simply a term; it carries a physiological meaning which does not explain the function(s) such solutes carryout (Bohnert and Shen; 1999).

Mechanisms of compatible solute includes maintenance of ion homeostasis and water relations, carbon/nitrogen partitioning, reserve allocation, or storage (and possibly diffusion) of reducing power (Bieleski, 1982; Blomberg and Adler, 1992;

Bohnert et al., 1995; Niu et al., 1995). There may be more than one function for a particular solute (Shen et al., 1997a, b), and, based on results from in vitro experiments (Smirnoff and Cumbes, 1989; Halliwell and Gutteridge, 1990; Orthen et al., 1994), also different compatible solutes may have different functions.

The main function of a compatible solute may be the stabilization of proteins, protein complexes or membranes under environmental stress. In in vitro experiments, compatible solutes at high concentrations have been found to reduce the inhibitory effects of ions on enzyme activity (Pollard and Wyn Jones, 1979; Yancey et al., 1982; Brown, 1990; Solomon et al., 1994). The addition of compatible solutes increased the thermal stability of enzymes (Back et al., 1979; Paleg et al., 1981; Galinski, 1993), and prevented dissociation of the oxygen-evolving complex of photosystem II (Papageorgiou and Murata, 1995).

The importance of compatible solute accumulation, interpreted as `osmotic adjustment', had been recognized long ago (Brown and Simpson, 1972; Borowitzka and Brown, 1974; Levitt, 1980). A correlation between compatible solute amount and tolerance has been documented (Storey and Wyn, 1977; Flowers and Hall, 1978; Bohnert et al., 1995 and references therein). Loss of turgor following water deficit caused either by lowering of water uptake through roots or continued evapotranspiration through stomata is very likely a signal for compatible solute synthesis, possibly through a pathway that is similar to the yeast high osmolarity glycerol osmotic signaling pathway (Shinozaki and Yamaguchi-Shinozaki, 1997).

Recent experiments addressed replacing the specific compatible solute found in one organism by a different compatible solute not normally present in that organism, preliminary results indicate that such simple replacement might not yield similar protection (Shen et al., 1998). Another idea discussed the subject of compatible solute is different compounds can function as compatible solutes.

Potassium, if available, serves this function in many unicellular organisms (Serrano, 1996) and sufficient potassium in the soil leads to more efficient exclusion of sodium in higher plants (Niu et al., 1995, and references therein). Also amino acids and some amino acid derivatives, sugars, acyclic and cyclic polyols, fructans, and quaternary amino and sulfonium compounds frequently act as compatible solutes (Levitt, 1980; McCue and Hanson, 1990; Delauney and Verma, 1993; Bartels and Nelson, 1994; Bohnert and Jensen, 1996 b).

Compatible solutes do not interfere with protein structure and function, and they alleviate inhibitory effects of high ion concentrations on enzyme activity. It is an osmo-regulatory function as osmolytes that is typically assigned to the multitude of compatible solutes which accumulate in response to osmotic stress (Bohnert and Shen; 1999). Some solutes, such as trehalose, do not respond to osmotic stress by accumulating to high amounts but are protective even at low concentrations (Mackenzie et al., 1988; Holmstro Èm et al., 1996). Glycine betaine (which may be present in high or low amounts), for example, protects thylakoid membranes and plasma membranes against freezing damage or heat destabilization (Coughlan and Heber, 1982; Jolivet et al., 1982; Zhao et al., 1992), indicating that the local concentration on membranes or protein surfaces may be more important than the absolute concentration.

2.3 Role of Glycine Betaine as osmo-regulator in corps:

GB application allowed Maize plants in the mildly-stressed treatment to overcome water limitation and continue growing which resulted in increased biomass relative to the untreated mildly stressed plants (Reddy et al; 2013). GB, could counteract the adverse effects of drought on wheat by improvement of growth vigor of root and shoot, leaf area, retention of pigments content, increasing the concentration of organic solutes (soluble sugars and soluble nitrogen) as osmo-protectants, keeping out the polysaccharides concentration and/or stabilization of essential proteins in both wheat cultivars, GB could improve the drought tolerance of both two wheat cultivars (sensitive, Sakha 94) and (resistant, Sakha 93) particularly the sensitive ones (Heshmat et al; 2011).

Exogenous application of GB on wheat cultivars as a pre-sowing seed treatment only improved the plant biomass. However, this increase in plant biomass could not be related to net CO2 assimilation rate. Of different Glycine Betaine levels used for pre-sowing seed treatment, 50 mM was found to be better than the other levels in affecting growth of wheat cultivars. (Mahmood et al; 2009) GB may provide protection against extreme temperature conditions and manipulation of biosynthetic pathway of glycine betaine into Soybean may be useful under stressful environments (Salem et al; 2005). Exogenous application of glycine betaine to Beans resulted in a favorable water status of plants as indicated by a slower decrease in leaf water potential and delayed wilting during water stress as compared to the untreated plants (Xing et al; 1999). The exogenous application of glycine betaine and shikimic acid improves the growth parameters of Sorghum plants by increasing the turgidity, stimulating leaf expansion and enhancing the production of photosynthetic pigments.

Therefore, the applied chemicals increase the yield capacity of Sorghum plants (Ibrahim and Aldesuquy; 2003).

Glycine betaine protection was more pronounced against irradiation stress at lower doses of γ-rays gamma-Irradiated on Fenugreek Plants, Post-treatment of irradiated seeds with glycine betaine partially alleviated adverse effects of radiation. Pre-treatment of seeds with glycine betaine may play an effective role in the radio-repair mechanism (Mossa and Jaleel; 2011). GB is a simple and useful cryo-protectant that works for wide range of Prokaryotic organisms under different cryopreservation regimens (Cleland et al; 2004). Intracellular accumulation of glycine betaine has been shown to confer an enhanced level of osmotic stress tolerance in Rhizobium melioti (Smith et al; 1988). Spraying with glycine betaine on corn at both stem elongation and pre flowering stages diminished detrimental effects of drought, Concentration of 150 ppm GB was superior to other concentration and pre flowering spraying was better than stem elongation. Therefore it could be suggest that in drought condition application of 150 ppm GB protects corn from detrimental effects of drought in similar agro-climate condition (Miri and Armin; 2013). CE (crude sugar beet extract) application is very useful as a commercial supplement in tomato production to improve growth and productivity during summer because it was more effective and less expensive than pure glycine betaine (Kanechi et al; 2013).

Natural source of GB (sugar beet extract) and pure GB were found to be equally effective in reducing the adverse effects of salt stress on Okra plants in terms of growth and some key physiological attributes (Habib et al; 2012). Glycine betaine levels increase readily during the cold acclimation of strawberry plants. Application of ABA to strawberry plants also triggers an increase in the glycine betaine levels and induces cold tolerance in the leaves. In addition, exogenous application of glycine betaine can induce cold tolerance in both unhardened and cold acclimating plants (Rajashekar et al; 1999). When Arabidopsis plants were subjected to water stress, the endogenous leaf glycine betaine level increased by about 18-fold over that in the control plants. When glycine betaine (10 mM) was applied exogenously to the plants as a foliar spray, the freezing tolerance increased from −3.1 to −4.5°C. Water stress lead to significant increase in the freezing tolerance, which was slightly less than that induced by the cold acclimation treatment. The results suggest that glycine betaine is involved in the induction of freezing tolerance in response to cold acclimation and water stress in Arabidopsis plants (Xinga and Rajashekar; 2001). Pre-soaking sorghum grains in Glycine betaine or pre-soaking plus spraying minimized the harmful effects of salinity on the ultrastructure of leaf mesophyll cells as compared to non-treated plants grown under saline conditions. However, GB applied as pre-soaking plus spraying showed the most beneficial effect in this respect (Arafa et al; 2009).

Foliar applications of GB at 0.5 and 1 mM ameliorating photo-inhibition and resulted in higher levels of CO2 assimilation rate, stomatal conductance, and transpiration rate in the three marigold cultivars namely, ‘Narai Yellow’, ‘Bali Gold’, and ‘Columbus Orange’ compared with those in the control under heat stress. Application of GB also resulted in lower levels of hydrogen peroxide, superoxide, lipid peroxidation, and cell death in all cultivars. The effect of GB on improving RWC (relative water content) was significant only in ‘Bali Gold’. In most cases, there were no significant differences between the effects of GB at 0.5 and 1 mM. Overall, these results indicate that the foliar application of GB could possibly be used to mitigate the effect of heat stress in marigold (Sorwong and Sakhonwasee; 2015).

GB applied at the vegetative growth stage was more effective in ameliorating the adverse effects of drought stress on tomato cv. PS. Due to GB-induced improvement in plant water status. The adverse effects of drought stress on tomato can be alleviated by the exogenous application of GB at different growth stages by modulating water relations. The decrease in yield of tomato fruit number and weight grown under drought conditions is largely due to the reduction in the number of flowering per plant and plant growth (Rezaei et al; 2012). Exogenous GB treatments mitigated the deleterious effects of salt stress in lettuce. Foliar GB treatments improved the growth of lettuce by alleviating salicylic acid (SC), water status, plant nutrient uptake, soluble sugar content and hormone content, moreover reducing membrane permeability (MP), lipid peroxidation (MDA) and H_2O_2, and helping plants to avoid Na toxicity under salinity conditions (Yildirim et al; 2015).

Glycine betaine (GB) accumulated in seeds of transformed tomato plants up to 1µmol g^{-1} dry weight (DW), while no detectable GB was found in wild-type (WT) seeds. Addition of GB to the germination medium or imbibition of seeds in a solution of GB enhanced the tolerance of WT seeds to high temperatures. GB, either applied exogenously or Accumulated in vivo in cod A-transgenic seeds, enhanced the expression of heat-shock genes in and improved the tolerance to high temperature of tomato seeds during germination (Shufen Li et al; 2011).

Chapter 3: Materials and Methods

3.1 Experimental Site:

The experiment was conducted during 2014-2015 growing season under natural conditions in a shade area ($8x5m^2$), with net intensity 60%, at Al-Foah Experimental Station (270N and 220S latitude and 510W and 570E longitude) of the College of Food and Agriculture, UAEU, Al Ain city, 160 km East of Abu Dhabi the capital city of United Arab Emirates.

3.2 Cultivation methods:

Alfalfa (*Medicago sativa* L.) & Cowpea (*Vigna unguiculata* L. Walp.) seeds were kindly provided by agricultural inputs commercial supplier “SHAT AL ARAB”. Seed sowing was carried out manually on (October 16^{th} 2014 for Alfa alfa 1g/pot) and on (December 1^{st} 2014 for Cowpea) 6 seeds/pot, in PVC cylinder pots (60 cm Height and 25 cm Diameter), volume of (65 liter), each cylinder contain, 10 cm of stones, 20 cm of sand and 25 cm of mix of sand and organic fertilizer (1:1).

The pots were irrigated as needed (agricultural farm recommended) 400 ml volume of tap water. Germination was on the third day in Alfa alfa and on the fourth day of sowing in Cowpea, after emergence 11 DAS (Days After Sowing), the seedlings were thinned to retain three seedlings in each pot, the second thinning was applied within a month to retain two seedlings for experiment. Seedlings were under natural light conditions; when the air temperature 24 – 15 °C day and night.

Figure 2: Field capture (plants Cowpea & Alfa alfa)

3.3 Application of Glycine betaine and Irrigation Treatments:

Alfa alfa & Cowpea were subjected to different concentrations of Glycine Betaine. The GB (Sigma-Aldrich, (98%) perchloric acid titration, (carboxymethyl) trimethyl ammonium inner salt, Oxyneurine, C5H11NO2, MW: 117.15, were purchased from Sigma Aldrich Co., and used for the present study. Glycine Betaine was applied in three different concentrations, 0 ppm (control), 100 ppm and 200 ppm. GB solution was prepared by mixed GB granular powder at a rate of 0.1 g /L of distilled water for the treatment 100 ppm and a rate of 0.2 g/L for the treatment 200 ppm, and applied manually over the top of the plant covering all over the plant's leaves, by using Pressurized Spray Bottle with 0.1% Tween 20 as surface spreader and applied for five times on the plants at 25, 30, 35, 40 and 45 DAS.

The water deficits factors were expressed at different irrigation intervals and fixed water quantity (400ml). The irrigation intervals were each 24 hours (WW - 100% - control), each 48 hours (WD - 60%) and each 72 hours (WD - 40%). Water was applied by controlled dripper irrigation system (Surface drip (DI) and subsurface drip irrigation (SDI) is most effective way to convey directly water and nutrients to plants and not only, does it save water but it also increases yields of vegetable crops (Tiwari et al., 1998; Tiwari et al., 2003).

Figure 3: Field capture (the experiment design)

For each treatment early morning, the plants were subjected for water deficit regimes 31 DAS or exactly after the second application of GB. The experiment had three levels for both variables: (Water irrigation regimes) and (Glycine Betaine concentrations). Consequently the research covered 9 treatments including the control units, in three replicates (pots) for each treatment in total 27 pots in three sets arranged for the research in Alfa alfa and the same arrangement for the Cowpea, the experimental was designed on CRBD (Completely Random Block design), as the sketches below:

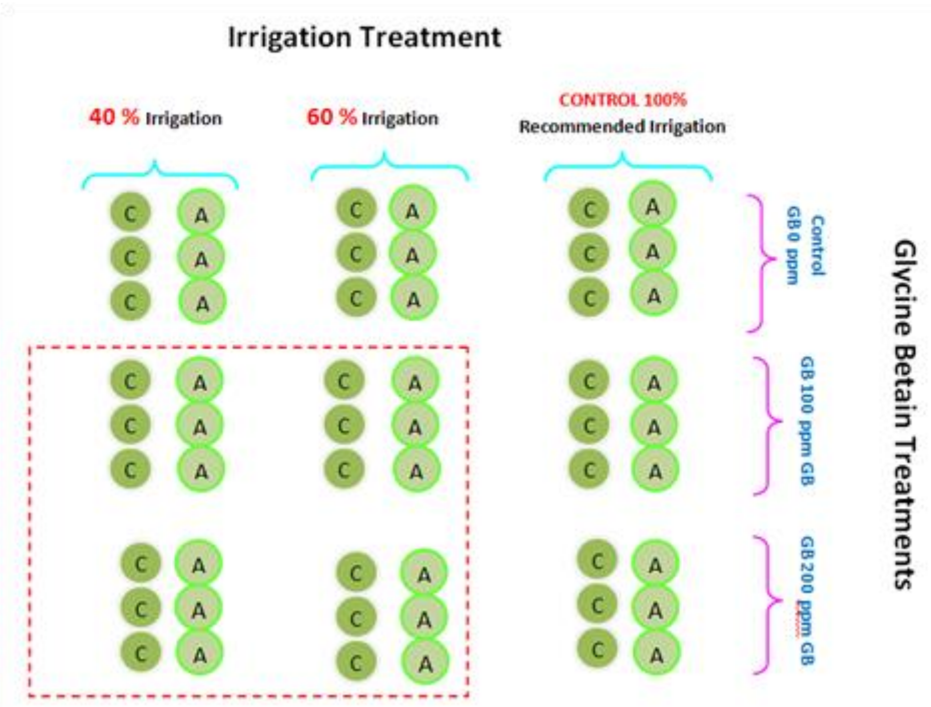

Figure 4: Sketches of the experiment

On the 159 DAS (days after sowing) or 68 DAT (days after treatments) in Alfa alfa, and on the 114 DAS or 68 DAT for Cowpea, plants were harvested for analysis.

3.4 Morphological parameters:

Shoot height and Root length:

Shoot height was measured from the soil level to the tip of the shoot and expressed in cm. the plant Root length was measured from the point of the first cotyledonary node to the tip of longest root and expressed in cm.

Shoot and Root fresh and dry weight:

After harvesting the plants were washed in the tap water, fresh weight for Shoot and Root was determined by using an electronic balance (Model – XK3190-A7M) and the values were expressed in grams. After taking fresh weight, the plants were dried at 75°C in hot air oven for 24 hours. After drying, the weight was measured and the values were expressed in grams.

Stem diameter (cm):

Stem diameter was measured for each plant by Vernier caliper and the values were expressed in mm.

Pods number:

The total number of pods, which were fully developed, were counted and expressed as number of pods per plant.

Pods fresh and dry weight:

Fresh weight of pods was determined by using an electronic balance (Model – XK3190-A7M) and the values were expressed in grams. After taking fresh weight, the pods were dried at 75°C in hot air oven for 24 hours. After drying, the weight was measured and the values were expressed in grams.

3.5 Physiological Parameters:

Pigments Analysis:

Estimation of Chlorophyll and Carotenoid content:

Chlorophyll and carotenoid were extracted from the leaves and estimated by the method of Arnon (1949).

500mg of fresh leaf material was ground with 10 ml of 80 per cent acetone at 4°C and centrifuged at 2500 rpm for 10 minutes at 4°C. This procedure was repeated until the residue became colourless. The extract was transferred to a graduated tube and made up to 10 ml with 80 per cent acetone and assayed immediately.

Three milliliters aliquots of the extract were transferred to a cuvette and the absorbance was read at 645, 663 and 480 nm with a spectrophotometer (U-2001-Hitachi) against 80 per cent acetone as blank. Chlorophyll content was calculated using the formula of Arnon and expressed in milligram per gram fresh weight.

Total chlorophyll (mg/ml) = $(0.0202) \times (A.645) + (0.00802) \times (A.663)$

Chlorophyll ‘a’ (mg/ml) = $(0.0127) \times (A.663) - (0.00269) \times (A.645)$

Chlorophyll ‘b’ (mg/ml)= $(0.0229) \times (A.645) - (0.00468) \times (A.663)$

Carotenoid content was estimated using the formula of Kirk and Allen (1965) and expressed in milligrams per gram fresh weight.

Carotenoid (mg/g) = $A.480 + (0.114 \times A.663 - 0.638 \times A.645)$

Biochemical analysis:

Estimation of Total Phenols content

Total phenol was estimated by the method of Malick and Singh (1980). 500 mg of fresh plant tissue was ground using a pestle and a mortar with 10 ml of 80% ethanol. The homogenate was centrifuged at 10 000 rpm for 20 minutes. The supernatant was evaporated to dryness. The residue was dissolved in 5 ml of distilled water and used as the extract. To 2 ml of the extract, 0.5 ml of Folin Ciocalteau reagent was added. After 3 min, 2 ml of 20% Na2 CO3 solution was mixed in throughly. The mixture was kept in boiling water for exactly 1 min and after cooling the absorbance was read at 650 nm. The total phenol was determined using a standard curve prepared with different concentrations of gallic acid.

Estimation of Proline:

Proline content was estimated following the method of Bates et al. (1973). Five hundred mg of plant material was taken in a pestle and mortar and homogenized with 10 ml of 3 per cent aqueous sulfosalicylic acid. Then the homogenate was filtered through what man No. 2 filter paper. The residue was re-extracted two times with 3 per cent sulfosalicylic acid and pooled. The filtrates were made up to 20 ml with 3 per cent sulfosalicylic acid and used for the estimation of proline.

Two ml of extract was taken in a test tube and 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid were added to it. The mixture was incubated for one hour at 100ºC in a water bath. The tubes were transferred to an ice bath to terminate the reaction. Then to each test tube 4 ml of toluene was added and mixed vigorously using a test tube stirred for 10-20 seconds. The toluene containing the chromophore was separated from the aqueous phase with the help of separating funnel and the absorbance was measured at 520 nm in a spectrophotometer using an appropriate blank. The proline content was determined from a standard curve prepared with proline and the results were expressed in milligram per gram dry weight.

Estimation of Glycine betaine

The samples were extracted and estimated following the method of Grieve and Grattan (1983). Five hundred mg of finely ground dried plant samples was mechanically shaken with 20ml of de-ionized water for 24hours at 25ºC. Time required for this step was determined by extracting the plant samples for 1, 4, 16, 24 and 48hours. The samples were then filtered and filtrates were stored in the freezer for analysis. Thawed extracts were diluted with 2N H_2SO_4 (1:1). The acid potassium tri-iodide solution for total QACs were prepared by dissolving 7.5g resublimed iodine and 10g potassium iodide in 1M HCI and filtered (Speed and Richardson, 1968). Precisely, 0.2ml of acid potassium tri-iodide reagent was added to an aliquot of sample containing between 10-15 μ g of QACs in water. The mixture was shaken and left for at least 90 minutes in an ice bath with intermittent shaking. Two ml of ice-cold water was added rapidly to the mixture to reduce the absorbance of blank and to improve replication. This was quickly followed by 10ml of 1, 2-dichloroethene in ice, and the 2 layers mixed well and kept at 4ºC (Storey and Wyn Jones, 1977). The absorbance of the lower organic layer was measured at 365nm in a Spectrophotometer. The results were expressed as glycine betaine equivalent by using glycine betaine for standard value.

Elemental Analysis:

Plant samples and immediately brought to the laboratory using plastic bags. The samples were air dried then oven dry at 105 ^{0}C for 3 hrs and the samples were grinded and stored in a desiccator for further analysis. The CEM Mars 5 microwave digestion system was used to extract the elements from the plants samples. The digestion procedure was based upon the recommendation in USEPA method 3015A guidelines. This microwave digestion method was designed to mimic extraction using conventional heating with nitric acid (HNO_3) and hydrochloric acid (HCL). The plant samples were prepared accurately by weighing 0.5 grams of sample into the microwave digestion vessels and adding 10ml of concentrated nitric acid (HNO3) and 2 ml hydrochloric acid (HCL) (Method 3015A, US Environmental Protection Agency, 2008). The vessels were capped and place in the microwave digestion system.

Varian ICP-OES model 710-ES simultaneous axially viewed plasma with full PC control of instrument settings and compatible accessories was used for the elemental analysis. For analysis a portion of homogeneous Plants samples are accurately weighed and treated with acids to destroy the organic matter and solubilized the recoverable elements. After cooling, the sample was made up to the volume with deionized water and filtered. The sample solution was aspirated through nebulizer and the resulting aerosol was transported to the plasma torch where excitation occurs. Element specific emission spectra were produced by radio-frequency inductively coupled plasma. The spectra were dispersed by a grating spectrometer, and intensities of the line spectra are monitored at specific wavelengths by a charged coupled detector. A fitted background correction was used to correct the blank signal and matrix effect.

The calibration blank was prepared by diluting 1 ml of concentrated nitric acid in 100 ml deionized water. Sufficient quantity was used to flush the system between standards and samples. The reagent blank contains the same volumes of all reagents

used in the processing of the samples. The reagents blank was carried thr complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.

3.6 Statistical Analysis:

The data pertained to all the characters studied were subjected to statistical analysis using SPSS-21.0 Version. The values were meant for three replicates of all the treatments and control. The calculated data expressed in Mean±SE.

Chapter 4: Results

The results of the present study are given in Tables 1 – 12 below:

Results of Morphology Parameters:

Table (1) shows the effects of GB under the water stress conditions on the Morphology Parameters in Cowpea

The effects of GB on the Shoot Height of cowpea was significantly higher in treatment of applying 100 ppm Glycine Betaine within 100% water, but it was reduced in the treatments of water stress both 40% and 60%, in treatments of combining Glycine Betaine with water stress the height was increased in treatments both 100 ppm & 200 ppm under drought stress of 60% of irrigation water.

Root Length behaves, it's found with applying of Glycine Betaine 100 ppm was increased, but it was reduced in 200 ppm of Glycine Betaine. When the plants exposed to the stress of water in 60% the length was significantly increased in Cowpea, and when the plants exposed to combined effects of Glycine Betaine and water stress the length of root was increased significantly in both 100 ppm & 200 ppm Glycine Betaine under drought stress 40 % of water irrigation.

In Cowpea the Fresh Shoot Weight was decreased in treatments of applying Glycine Betaine and treatments of water stress and the combination of them.

In the treatments of water stress and applying Glycine Betaine and their combinations on Shoot Dry Weight of Cowpea the observations was reduced in all the treatments comparing with control.

The effects on Root Fresh Weight induced by applying Glycine Betaine within the water stress in Cowpea was reduced the fresh weight of the root within the treatments of applying Glycine Betaine when it compared with the control. In treatments of combining applying Glycine Betaine under the drought stresses it shown the increasing in Root fresh weight in both treatments of Glycine Betaine 100 ppm & 200 ppm within the stress of 40% of irrigation water in Cowpea.

In the Root Dry weight the Glycine Betaine application within the conditions of drought stress in Cowpea the observation it’s found that the dry weight of the root was reduced in all treatments when it compared with the control.

In the effect of the Glycine Betaine application under drought water stress on the Cowpea Stem Diameter was reduced by both application of Glycine Betaine 100 ppm & 200 ppm comparing with the control, on contrast the stem diameter was increased under water stress of drought significantly, but when combining the application of Glycine Betaine with in the conditions of water stress the diameter of stem was increased significantly in treatment of applying 100 ppm & 200 ppm GB in 60% of water irrigation.

The Number of pods in the Cowpea has been effected by the application of Glycine Betaine within the drought conditions, its increased in the application of Glycine Betaine 100 ppm and increased significantly in the treatment of combining the 200 ppm with 60% irrigation water but increased highly in the treatment of combining the applying of 100 ppm Glycine Betaine with giving 60% of water irrigation.

Fresh Weight of Pods in Cowpea has been effected by the Glycine Betaine application within drought conditions, it was reduced in the treatments of applying Glycine betaine both 100 ppm & 200 ppm and in treatments of water stress, and in the combination of Glycine Betaine treatments within the water stress conditions when it compared with the control plants.

The effect of Glycine betaine, drought stress and their combination induced changes on Pods Dry Weight of Cowpea. In applying 100% of GB with 60% of water stress increased the pods dry weight of cowpea significantly when compared to control.

Table (2) shows the effects of GB under the water stress conditions on the Morphology Parameters in Alfa alfa.

The effects of GB on the Shoot Height of Alfa alfa was increasing the height in both concentrations 100 ppm & 200 ppm of Glycine Betaine, but the height was reduced in the treatments of water stress both 40% and 60%, in combining the effects of GB under drought stress conditions the height was increased in treatment 100 ppm GB under 60% of irrigation water.

Root Length of Alfa alfa was increased with the applications of GB both 100 ppm & 200 ppm, when the plants were exposed to the stress of water drought conditions, the root length was increased significantly within 60% of irrigation water. With the applications of GB under water drought stress conditions the plants were decreased its root length.

The Shoot Fresh Weight in Alfa alfa was increased in treatment 100 ppm of Glycine Betaine, but it was reduced in treatments of water stress and treatments of combining the Glycine Betaine application under the water stress conditions comparing with the control.

In Alfa alfa the Shoot Dry Weight was highly increased in all treatments on the plants and the highest increment it was double of the weight in treatment of applying 200 ppm of Glycine Betaine when it compared with control. In treatments of combining Glycine Betaine and water stress it found increasing in all treatm

and the highest increased was in treatments of 100 ppm Glycine Betaine within 40% of irrigation.

The Root Fresh Weight in Alfa alfa was increased within the application of 200 ppm concertation of Glycine Betaine comparing with the control, but increased intensively in both treatments 40% & 60% of water drought stress. The root fresh weight was increased in treatments of combining application of Glycine Betaine and water drought stress in 200 ppm of GB within 40% & 60% of irrigation water.

In Alfa alfa the Root Dry Weight was increased in all the treatments and the highly increasing was found in application of 200 ppm of Glycine Betaine within the drought stress of water irrigation 40% and 60%.

Table 1. Glycine betaine, water stress and combined effects on the Morphology Parameters in Cowpea

Parameter	Control	GB		Water Stress		GB+ Water Stress			
	0 ppm of GB+ 100% water	100 ppm	200 ppm	40%	60%	100ppm + 40%	100ppm + 60%	200ppm + 40%	200ppm + 60%
SH (cm)	123.08±11.59a	125.75±6.18a	121.75±4.83a	37.67±1.53e	116.08±3.04c	75.75±10.33d	137.5±6.29b	87.75±4.78d	142.08±7.26b
RL (cm)	16.08±1.23a	17.67±0.83c	15.33±0.65b	15.67±0.3b	18.67±2.4c	16.50±1.13a	13.17±0.6e	20±0.87d	15.92±0.96b
SFW (g)	77.55±4.89a	64.93±0.93b	63.11±4.95b	34.13±2.67d	47.23±1.52c	45.67±5.87c	59.64±4.55b	47.91±5.23c	62.21±0.41b
SDW (g)	12.43±0.86a	11.01±0.77b	10.75±0.97b	8.14±0.98c	7.26±0.25c	5.38±0.54e	9.05±0.66c	7.19±0.83c	9.53±0.28c
RFW (g)	2.96±0.48a	1.52±0.1d	1.52±0.19d	2.29±0.21b	2.05±0.17c	3.23±0.27b	2.21±0.16b	3.26±0.29b	2.33±0.14c
RDW (g)	1.16±0.2a	0.77±0.02c	0.93±0.08b	0.75±0.01c	0.53±0.07d	0.83±0.06b	1.08±0.17a	0.86±0.1b	0.86±0.04c
SD (mm)	0.55±0a	0.48±0.02b	0.45±0c	0.6±0b	0.55±0.03a	0.52±0.02b	0.55±0a	0.52±0.02b	0.58±0.02a
NP	12.1±1.25a	14.0±0.76b	11.50±0.29b	7.17±0.67d	12.33±0.33b	9.83±0.67c	15.50±1.73c	9.17±0.6c	12.83±0.67a
PFW (g)	19.63±0a	13.39±0.1c	12.29±2.19d	8.07±1.17e	15.1±0.79c	10.37±1.72c	17.22±0.46b	12.39±2.82d	15.96±0.88c
PDW (g)	17.3±0.6a	11.97±0.33c	7.47±1.05d	4.51±0.29e	12.24±0.49c	5.95±0.4e	14.21±1.27b	6.50±0.32e	12.08±1.02c

(Values are the mean of three replicates) – (SH: Shoot Height), (RL: Root Length), (SFW: Shoot fresh weight), (SDW: Shoot dry weight), (RFW: Root fresh weight), (RDW: Root dry weight), (SD: Stem diameter), (NP: Number of Pods), (PFW: Pods Fresh Weight), (PDW: Pods Dry Weight)

Table 2. Glycine betaine, water stress and combined effects on Morphology Parameters in Alfa alfa

Parameter	Control	GB		Water Stress		GB+ Water Stress			
	0 ppm of GB+ 100% water	100 ppm	200 ppm	40%	60%	100ppm + 40%	100ppm + 60%	200ppm + 40%	200ppm + 60%
SH (cm)	47±0.58a	56.67±0.88b	58.33±2.91b	25±1.15e	46.67±0.33c	33.67±1.86d	56±2.31b	30±0.58d	46±1.53c
RL (cm)	29.0±0.58a	35.67±1.86b	32.0±2.08b	26.33±0.88c	30.67±0.88a	27.67±0.33c	27.33±0.67c	26.33±1.45 c	26.0±1.0c
SFW (g)	181.61±15.04 a	205.94±15.98 b	145.41±10.53 c	111.62±5.10 d	152.26±13.83 b	124.44±5.46 c	144.52±18.81 c	54.4±5.93e	60.0±10.33 e
SDW (g)	25.71±2.18a	28.56±0.35b	50.79±0.62d	28.98±1.29c	32.06±1.53c	45.43±3.65d	48.55±9.19d	41.74±1.92 d	38.42±2.42 d
RFW (g)	37.88±2.16a	33.10±4.35b	48.16±8.48c	58.32±1.64d	80.55±8.89e	33.53±4.01b	45.61±1.12a	77.74±2.92 e	70.87±1.62 d
RDW (g)	13.01±0.97a	17.83±2.26b	33.57±5.4c	21.05±0.16c	27.21±1.47a	18.93±3.7b	21.05±0.16c	49.11±1.87 d	42.65±0.76 d

(Values are the mean of three replicates) – (SH: Shoot Height), (RL: Root Length), (SFW: Shoot fresh weight), (SDW: Shoot dry weight), (RFW: Root fresh weight), (RDW: Root dry weight)

Physiology Parameters:

Pigments Contents: Chlorophyll ‘A’, Chlorophyll ‘B’, Total Chlorophyll and Carotenoid

Table (3) shows the effects of GB under the drought water stress conditions induced changes on the pigments content of Chlorophyll ‘A’, Chlorophyll ‘B’, Total Chlorophyll and Carotenoid in Cowpea.

All pigments content in Cowpea have been affected and raised obviously by the of applications of Glycine Betaine both 100 ppm & 200 ppm, but its reduced in the treatments of drought stress 60% and 40% of irrigation water. And in the treatments of combining applying Glycine Betaine under the water stress conditions it shown that the pigments content reduced in all treatments and Chlorophyll ‘A’ has been reduced slightly in treatments of 200 ppm & 100 ppm of 40% of irrigation water in Cowpea.

Table (4) shows the effects of GB under the drought water stress conditions induced changes on the pigments content of Chlorophyll ‘A’, Chlorophyll ‘B’, Total Chlorophyll and Carotenoid in Alfa alfa.

In the effects of applying Glycine Betaine under the drought stress conditions on pigments content, Chlorophyll ‘A’, Chlorophyll ‘B’, Total Chlorophyll and Carotenoid in Alfa alfa, it’s found that the applications of Glycine Betaine both 100 ppm & 200 ppm are induce to increase pigments content in Alfa alfa, but with the treatments of drought stress and the treatments of combining the drought stress with Glycine Betaine application the pigments content were decreased.

Table 3. Glycine betaine, water stress and combined effects on Pigments Content Chlorophyll ‘A’, ‘B’, Total Chlorophyll and Carotenoid (mg/g) in Cowpea

Parameter	Control	GB		Water Stress		GB+ Water Stress			
	0 ppm of GB+ 100% water	100 ppm	200 ppm	40%	60%	100ppm + 40%	100ppm + 60%	200ppm + 40%	200ppm + 60%
Chlo. A	0.11±0.01a	0.13±0.0b	0.13±0.02b	0.07±0.01d	0.09±0.01c	0.09±0.02c	0.08±0.01c	0.10±0.01a	0.09±0.01b
Chlo. B	0.04±0.01a	0.05±0.0b	0.04±0.01a	0.02±0.0d	0.03±0.0c	0.03±0.01c	0.03±0.0c	0.03±0.0c	0.03±0.0c
Total Chlo.	0.15±0.03a	0.19±0.01c	0.17±0.03b	0.10±0.01e	0.12±0.01d	0.11±0.03d	0.11±0.01d	0.13±0.02b	0.12±0.01c
Carotenoid	0.55±0.09a	0.64±0.05c	0.62±0.09b	0.37±0.00d	0.47±0.04b	0.43±0.11c	0.42±0.05c	0.48±0.05b	0.45±0.05c

(Values are the mean of three replicates) – (Chlo.A: Chlorophyll ‘A’), (Chlo.B: Chlorophyll ‘B’), (Total Chlo.: Total Chlorophyll) and Carotenoid

Table 4. Glycine betaine, water stress and combined effects on Pigments Content Chlorophyll 'A', 'B', Total Chlorophyll and Carotenoid (mg/g) in Alfa alfa

Parameter	Control	GB		Water Stress		GB+ Water Stress			
	0 ppm of GB+ 100% water	100 ppm	200 ppm	40%	60%	100ppm + 40%	100ppm + 60%	200ppm + 40%	200ppm + 60%
Chlo. A	5.40±0.17a	6.0±0.43b	6.61±0.09c	2.82±0.23d	4.77±0.19c	2.83±0.21d	4.78±0.08c	2.71±0.11e	4.65±0.37c
Chlo. B	2.02±0.13a	2.21±0.14b	2.51±0.12c	0.78±0.08e	1.70±0.06d	0.80±0.06e	1.76±0.11d	0.79±0.04e	1.81±0.08d
Total Chlo.	7.42±0.27a	7.36±0.33a	9.13±0.21c	3.6±0.31d	6.46±0.25b	3.63±0.27d	6.54±0.08b	3.50±0.15d	6.46±0.29b
Carotenoid	0.37±0.01a	0.40±0.04b	0.42±0.03b	0.16±0.02e	0.27±0.01d	0.16±0.01e	0.26±0.01d	0.14±0.01d	0.31±0.02c

(Values are the mean of three replicates) – (Chlo.A: Chlorophyll 'A'), (Chlo.B: Chlorophyll 'B'), (Total Chlo.: Total Chlorophyll) and Carotenoid

Biochemical Analysis:

Proline concentration:

In Tables (5) & (6) shows the Proline concentration was decreased in the treatments of applying the Glycine Betaine and in the water regimes treatments and in their combination, in both plants Cowpea and Alfa alfa.

Phenols content:

The phenols content in the leaves of Cowpea it shown in Table (7) that was reduced in the treatments of applying Glycine Betaine, and in treatment of drought stress its increased in 60% of the irrigation water also its increased in the combination of treatments of Glycine Betaine 100 ppm within the drought stress of 60% of the irrigation water.

In Alfa alfa the application of Glycine Betaine 200 ppm was induced the phenols content in the plants and also when it combine the application of Glycine Betaine 100 ppm & 200 ppm with the drought stress treatments of 40% & 60% Its increased the phenol levels in the leaves except the treatment of 100 ppm with 60% of the irrigation water the phenols level was decreased, Table (8).

Table 5. Effect of Glycine betaine, drought stress and their combination induced changes on Proline content (mg/g) of Cowpea

Plant	Control	GB		Water Stress		GB+ Water Stress			
	0 ppm of GB+ 100% water	100 ppm	200 ppm	40%	60%	100ppm + 40%	100ppm + 60%	200ppm + 40%	200ppm + 60%
Cowpea	1.08±0.14[a]	0.80±0.08[b]	0.97±0.23[b]	0.58±0.09[c]	0.50±0.06[d]	0.56±0.10[d]	0.68±0.17[c]	0.78±0.12[c]	0.66±0.07[d]

(Values are the mean of three replicates)

Table 6. Effect of Glycine betaine, drought stress and their combination induced changes on Proline content (mg/g) of Alfa alfa

Plant	Control	GB		Water Stress		GB+ Water Stress			
	0 ppm of GB+ 100% water	100 ppm	200 ppm	40%	60%	100ppm + 40%	100ppm + 60%	200ppm + 40%	200ppm + 60%
Alfa alfa	4.74±0.03[a]	4.46±0.05[b]	4.38±0.06[b]	1.18±0.05[c]	4.40±0.03[b]	0.76±0.05[d]	4.36±0.01[b]	0.94±0.05[d]	4.41±0.02[b]

(Values are the mean of three replicates)

Table 7. Effect of Glycine betaine, drought stress and their combination induced changes on Phenol content (mg/g) of Cowpea

Plant	Control	GB		Water Stress		GB+ Water Stress			
	0 ppm of GB+ 100% water	100 ppm	200 ppm	40%	60%	100ppm + 40%	100ppm + 60%	200ppm + 40%	200ppm + 60%
Cowpea	0.34±0.07^{a}	0.31±0.03^{b}	0.27±0.02^{c}	0.01±0.00^{e}	0.37±0.03^{b}	0.01±0.00^{e}	0.35±.0.02^{b}	0.03±0.01^{d}	0.32±0.03^{c}

(Values are the mean of three replicates)

Table 8. Effect of Glycine betaine, drought stress and their combination induced changes on Phenol content (mg/g) of Alfa alfa

Plant	Control	GB		Water Stress		GB+ Water Stress			
	0 ppm of GB+ 100% water	100 ppm	200 ppm	40%	60%	100ppm + 40%	100ppm + 60%	200ppm + 40%	200ppm + 60%
Alfa alfa	0.35±0.02^{a}	0.28±0.01^{c}	0.39±0.08^{c}	0.37±0.03^{b}	0.28±0.01^{d}	0.47±0.04^{c}	0.31±0.02^{c}	0.52±0.08^{e}	0.39±0.01^{b}

(Values are the mean of three replicates)

Glycine Betaine Levels:

In Table (9) Glycine Betaine contents has been increased in the plants Cowpea in all the treatments of applying Glycine Betaine and in treatments of water drought stress and in the combination of Glycine Betaine applications within the waters stress treatments.

In Alfa alfa, Table (10) the Glycine Betaine content was reduced when it’s applied exogenously as a foliar spray to the leaves. In the treatments of drought stress the Glycine Betaine levels were rise up in both 40% & 60% of irrigation water. And in the combining the exogenous applying for Glycine Betaine with the drought stress conditions the levels of Glycine Betaine were increased in all treatments of 100 ppm & 200 ppm within 40% & 60% of irrigation water, except the treatments of 60% irrigation with 200 ppm the Glycine Betaine content was slightly reduced.

Table 9. Effect of Glycine betaine, drought stress and their combination induced changes on Glycine Betaine content (mg/g) of Cowpea

Plant	Control	GB		Water Stress		GB+ Water Stress			
	0 ppm of GB+ 100% water	100 ppm	200 ppm	40%	60%	100ppm + 40%	100ppm + 60%	200ppm + 40%	200ppm + 60%
Cowpea	56.12±1.62^{a}	59±0.55^{b}	71.43±2.44^{d}	63.95±5.73^{c}	60.99±3.26^{c}	58.65±1.18^{b}	59.43±1.16^{c}	60.33±0.82^{c}	55.58±1.54^{b}

(Values are the mean of three replicates)

Table 10. Effect of Glycine betaine, drought stress and their combination induced changes on Glycine Betaine content (mg/g) of Alfa alfa

Plant	Control	GB		Water Stress		GB+ Water Stress			
	0 ppm of GB+ 100% water	100 ppm	200 ppm	40%	60%	100ppm + 40%	100ppm + 60%	200ppm + 40%	200ppm + 60%
Alfa alfa	45.41±0.35^{a}	44.52±1.84^{b}	44.87±5.60^{b}	62.16±2.53^{e}	51.21±2.94^{c}	61.19±7.69^{e}	43.89±1.59^{b}	58.77±1.66^{d}	42.41±1.80^{c}

(Values are the mean of three replicates)

Mineral Nutrients content:

Calcium, Copper, Iron, Potassium, Magnesium, Manganese, Sodium, Phosphorus, Sulfur and Zinc

In the Table (11), it shows the effect of applying the Glycine Betaine under the water drought stress conditions on the Mineral Nutrients contents in the Cowpea.

Calcium content showed high increased under the water stress treatments and in treatments of applying exogenously Glycine Betaine and also in treatments of combining both effects of drought stress 60% and 40% of irrigation water within application of the foliar spray Glycine Betaine and the higher increased were in treatments 200 ppm within 60% of the irrigation water.

Drought stress caused decreased in the Copper content in Cowpea in water stress treatments, but in the combination of the Glycine Betaine application treatments with the drought stress treatments, it’s increased the Copper content in cowpea in treatment 100 ppm of Glycine Betaine with 40% of water comparison to the control.

The effect of Glycine Betaine in the conditions of drought stress cause increasing the Iron content in Cowpea plants in all the treatments, and the plants under stress of water 40% & 60% of irrigation water were induced to have higher content of Iron.

In Cowpea the Glycine Betaine application induced the Potassium content in both 100 ppm & 200 ppm. In treatments of water stress the Potassium content increased in 60% of irrigation water. In treatments of combining the drought stress and application of Glycine Betaine it increased the Potassium content in all treatments and it was the higher content of Potassium in treatment 100 ppm of GB with 40% of water comparison with the control.

Glycine Betaine applications were induced increased in Magnesium content in Cowpea in both treatments 100 ppm & 200 ppm when it compared with the control. Within treatments of water stress the Magnesium level was increased in stress of 60% of water irrigation. The treatments of combining the effect of Glycine Betaine on the drought stress the Magnesium content was increased in all treatments and was the higher in applying 100 ppm in 40% of water irrigation.

The effects of the treatments of applying the Glycine Betaine and water stress and their combinations on the Manganese content in Cowpea was, In treatment of applying Glycine Betaine 200 ppm the Manganese content was increased compared with control. Under water drought stress treatment the Manganese content was increased highly in treatment of 40% of irrigation water.

In combining the applying of Glycine Betaine under water stress the Manganese content in Cowpea was increased significantly in all the treatments except in treatment 200 ppm of Glycine Betaine under 40% of water the concentration of Manganese was decreased.

The effects of Glycine Betain , drought stress and their combinations on the Phosphorus content in Cowpea and it was reduced within the drought stress and within drought stress combining with Glycine Betaine applying on the plants.

The sulfur content was affected by the experiment, the drought induced to increase the Sulfur level in Cowpea in both 40% and 60% of water deficit. In treatments of Glycine Betaine the sulfur level was increased highly in 100 ppm. In combination of drought stress treatments with applying Glycine Betaine the Sulfur content was increased in all the treatments except 100 ppm Glycine Betaine and 60% of irrigation water was decreased.

The Zinc content was increased with the treatment 100 ppm of Glycine Betain in the Cowpea plants. Within the treatments of drought stress the Zinc level increased slightly in treatment 40% of water. The combination treatments of Glycine Betain with drought stress the level of Zinc were decreased.

Table 11. Effect of Glycine betaine, drought stress and their combination induced changes on Mineral Nutrients mg/Kg (ppm) content of Cowpea

	Control	GB		Water Stress		GB+ Water Stress			
Elem.	0 ppm of GB+ 100% water	100 ppm	200 ppm	40%	60%	100ppm + 40%	100ppm + 60%	200ppm + 40%	200ppm + 60%
Ca	2.9±0.06a	3.47±0.18b	4±0.11c	3.73±0.26c	3.03±0.27b	3.07±0.12b	3.17±0.19b	2.43±0.12c	3.77±0.25c
Cu	5.45±0.52a	4.61±0.31b	4.34±0.17b	4.81±0.04b	3.65±0.02c	6.19±1.06b	4.17±0.18b	3.58±0.18c	3.87±0.07c
Fe	0.02±0.0a	0.02±0.0a	0.01±0.0b	0.04±0.02d	0.04±0.0d	0.03±0.01c	0.03±0.0c	0.03±0.0c	0.04±0.01d
K	0.84±0.08a	1.09±0.05c	1.08±0.09c	0.77±0.02b	0.95±0.1b	1.37±0.03e	1.04±0.11d	1.1±0.06d	1.2±0.12e
Mg	0.66±0.02a	0.85±0.01c	0.84±0.02c	0.12±0.01d	0.84±0.05c	0.9±0.03d	0.77±0.01b	0.85±0.08c	0.84±0.05c
Mn	46.65±5.23a	41.69±5.06	64.66±2.41	90.19±11.21	35.25±2.15	55.84±4.72	51.50±1.38	38.40±1.85	60.13±4.86
P	0.34±0.04a	0.32±0.02b	0.3±0.02c	0.25±0.01d	0.18±0.01e	0.24±0.03d	0.31±0.02b	0.14±0.01e	0.26±0.01d
S	0.33±0.03a	0.50±0.05d	0.31±0.0b	0.43±0.03c	0.37±0.02c	0.47±0.03d	0.31±0.02b	0.51±0.05e	0.36±0.01b
Zn	92.44±8.76a	105.49±14.89c	65.54±2.60c	93.61±3.68b	62.22±2.42c	88.71±8.27b	43.32±2.77e	65.63±2.97d	53.93±7.12c

(Values are the mean of three replicates)

In the Table (12), it shows the effect of applying the Glycine Betaine under the water drought stress conditions on the Mineral Nutrients contents in the Alfa alfa.

Calcium levels were decreased within the drought treatments in Alfa alfa and also reduced with the treatments of applying Glycine Betaine. In the treatments of combining the drought stress and the applying for Glycine Betaine the Calcium level was reduced but it reduced slightly in treatment of 100 ppm within 60% of irrigation water.

In Alfa alfa the application of Glycine Betaine slightly decreased the Copper content both 100 ppm and 200 ppm, and in drought stress treatments the copper level were decreased, in treatments of combination the Glycine Betaine exogenous with water stress the copper content increased in 100 ppm and 40% treatment.

In Alfa alfa the drought stress reduced the Iron level, also the treatments of combining the water stress with Glycine Betaine applications were reduced the Iron content.

Water drought stress conditions and the Glycine Betaine treatments were reduced the potassium content in the Alfa alfa.

In Alfa alfa the Magnesium content was decreased with applying Glycine Betaine and within the drought stress, in treatment of combining the drought stress effects 60% with applying 100 ppm of Glycine Betaine the Magnesium content was decreased slightly compering with the control.

In Alfa alfa the Manganese content when compared with control, was decreased by the applying of Glycine Betaine and under water stress treatments and in combining the Glycine Betaine application with the drought stress.

In Alfa alfa the Sodium content was increased when the plant exposed to Glycine Betaine both 100 ppm & 200 ppm when it compared with the control. Under water drought stress conditions the Sodium content was increased within the stress of 40% of irrigation water. In treatments of combining the applications of Glycine Betaine on drought stress the Sodium content was increased in all treatments 100 ppm & 200 ppm in both 60% & 40% of water.

In Alfa alfa crop the Sulfur content was increased with the application of Glycine Betaine in both 100 ppm & 200 ppm. The drought stress conditions were induced reduction in sulfur level in treatment 60% of water. The application of Glycine Betaine within the drought stress was enhancing the Sulfur level in all treatments except the treatment 200 ppm Glycine Betaine with 40% of water the sulfur content was decreased.

In Alfa alfa the level of Zinc were decreased in Glycine Betaine application. With drought stress treatment 60% it's induced to rise up the Zinc content in plants. In combination of Glycine Betain and water stress the Zinc level decreased in 200 ppm with both treatments of drought stress but in treatment of 100 ppm of Glycine Betain with drought stress both 40% & 60% the level of Zinc were increased and the treatment 100 ppm with 60% was the higher increased.

Table 12. Effect of Glycine betaine, drought stress and their combination induced changes on Mineral Nutrients content mg/Kg (ppm) of Alfa alfa

	Control	**GB**		**Water Stress**		**GB+ Water Stress**			
Elem.	0 ppm of GB+ 100% water	100 ppm	200 ppm	40%	60%	100ppm + 40%	100ppm + 60%	200ppm + 40%	200ppm + 60%
Ca	4.10±0.46a	3.77±0.12b	3.20±0.06c	2.37±0.09d	3.77±0.09b	3.07±0.09c	3.53±0.12b	3.0±0.00c	2.83±0.15c
Cu	15.70±0.94a	15.39±0.69b	15.52±1.13b	12.77±0.85c	15.10±0.81b	13.73±0.29c	17.83±0.20c	11.63±0.74d	12.68±0.39c
Fe	0.30±0.03a	0.25±0.01b	0.26±0.03b	0.17±0.00c	0.25±0.23b	0.16±0.02d	0.29±0.01d	0.18±0.01c	0.16±0.02c
K	0.90±0.03a	0.89±0.03b	0.74±0.03d	0.78±0.04c	0.79±0.05c	0.750.05d	0.68±0.01d	0.60±0.00e	0.62±0.02e
Mg	1.36±0.22a	0.95±0.03c	0.01±0.06e	0.74±0.02d	0.89±0.45c	0.83±0.07d	1.21±0.07b	0.78±0.00c	0.74±0.04d
Mn	106.61±7.61a	71.86±2.12	74.00±3.62	52.64±0.73	88.65±6.41	71.87±5.51	88.86±5.15	59.33±2.22	57.06±1.94
Na	0.32±0.02a	0.37±0.03b	0.44±0.01c	0.39±0.04b	0.29±0.03c	0.35±0.03b	0.38±0.01b	0.48±0.01d	0.48±0.03d
S	0.19±0.00a	0.23±0.01b	0.21±0.01b	0.19±0.1b	0.16±0.1c	0.21±0.00b	0.22±0.0b	0.17±0.00c	0.19±0.01b
Zn	125.36±11.36a	115.98±8.18b	107.67±8.93c	121.23±6.90b	166.66±7.24d	126.67±4.78b	183.41±7.10e	88.15±3.57d	111.45±3.45c

(Values are the mean of three replicates)

Chapter 5: Discussion

The present study was objective to determine the effect of two inverse factors exogenous application of Glycine Betaine and drought stress, affecting the growth and performance of forage crops Cowpea and Alfa alfa. Herewith the discussion of the results obtain from the experiment in morphology growth, pigments levels, biochemical and metals contents.

Shoot length in Cowpea was decreased under drought stress conditions treatments when compared with the control. While with application of exogenous Glycine Betaine individually and with drought stress, it's induced to increase the shoot height in treatment 100 ppm application of Glycine Betaine and in treatments of both 100 ppm & 200 ppm under drought stress of 60% of irrigation water. The root length in Cowpea was increased under drought stress in treatment 60% of water. In application of Glycine Betaine 100 ppm & 200 ppm with drought stress 40% of irrigation water the root length was increased compering with the control. Shoot height and root length values increased significantly by exogenous application of GB ($p \leq 0.05$) in tomato reported by (Rezaei et al., 2012).

In Alfa alfa the shoot height within drought stress was declined. With application of Glycine Betaine the shoot height was increased significantly, in drought stress treatments with applying Glycine Betaine the shoot height was decreased except the treatment of 60% of water with 100 ppm of Glycine betaine was increased the shoot height, (Reddy et al., 2013) was reported plant height declined with water deficit and was affected by GB application only under WD60 conditions, exhibiting a 14% increase in height with Glycine Betaine application. Root length in Alfa alfa was increased in treatments of applying Glycine Betaine, as reported by (Rezaei et al., 2012).while the root length was decreased significantly within combination treatments of drought stress and applications of Glycine Betaine. Measured growth characteristics declined linearly with increased water deficit intensity, both with and without GB application in maize, reported by (Reddy et al., 2013).

Drought stress inhibited the shoot growth significantly in Cowpea and Alfa alfa studied. Similar results were observed in avocado (Chartzoulakis *et al*., 2002), soybean (Ohashi *et al*., 2002), Okra (Bhatt and Srinivasa Rao, 2005), Pearl millet (Kusaka *et al.,* 2005), *Populus* species (Yin *et al*., 2005), *Abelmoschus esculentus* (Sankar *et al*., 2007), olive (Bacelar *et al*., 2007) and *Petroselinum crispum* (Petropoulos *et al.,* 2008). Decrease in shoot length may prevent excess water loss by reducing number of active stomata and transpiration rate. Drought stress increased the root length in *Eucalyptus microthea* seedlings (Li *et al*., 2000), *Populus* species (Yin *et al*., 2005), sunflower (Manivannan *et al*., 2007), *Cannabis sativa* (Amaducci *et al*., 2008), Oak species (Rodriguez-calcerrada *et al*., 2008), Parsley (Petropoulos *et al*.,2008) and *Triticum aestivum* (Dickin and Wright 2009). The development of root system may increases the water uptake under drought stress.

In cowpea the shoot fresh and dry weight was significantly decreased within water stress treatments and within application of Glycine Betaine. Shoot biomass in beans decreased in 32% in response to water stress compared to untreated control plants, Glycine Betaine treatments in plants subjected to long term water stress showed little or no effect in shoot biomass, as reported by (Xing & Raja shkar, 1999), water deficit reduced growth criteria (shoot fresh weight, shoot dry weigh, leaf area) significantly, as reported by (A.H. Ibrahim & H.S. Al desuquy; 2002). In Alfa alfa the shoot fresh weight was decreased under drought stress with or without exist of Glycine Betaine except with treatment of individually applying Glycine Betaine 100 ppm it increased the fresh weight of the plan significantly. But the shoot dry weight was increased highly in conditions of drought stress with and without applications of Glycine betaine. Total leaves fresh weight decreased under drought stress and total leaves dry weight increased significantly in drought conditions and by exogenous application of Glycine Betaine, reported by (Rezaei et al., 2012).

A decrease in total dry matter may be due to the considerable decrease in plant growth, photosynthesis and canopy structure, as indicated by leaf senescence during water stress in *Abelmoschus esculentum* (Sankar *et al*., 2007). Changing resource pools (e.g., water or nutrient availability) may also affect the distribution of biomass in sunflower (Villalobos and Ritchie, 1992), rice seedlings (Wang and Liang, 1995; Wang and Quinn, 2000) and sunflower (Soriano *et al.,* 2004). Drought stress decreases both the relative variation in plant biomass and the concentration of mass within a small fraction of the population. This is supported by earlier studies in *Quercus rubra* (Weber and Gates, 1990) and sorghum (Yadav *et al.,* 2005).

In cowpea the root fresh weight was increased within the higher water stress 40% of irrigation water compared with control, increased root growth was reported by (Tahir et al., 2003) in mango tree under water stress. But the root dry weight was decreased within the drought stress and in applications of Glycine Betaine, (A.H. Ibrahim & H.S. Aldesuquy; 2002)

Decreased total dry weight may be due to the considerable decrease in plant growth, photosynthesis and canopy structure as indicated by leaf senescence during drought stress in *Abelmoschus esculentum* (Bhatt and Srinivasa Rao, 2005); *Poplar* species (Jian Ren *et al*., 2007); *Ricinus communis* (schurr *et al*., 2000), *Vicia faba* (Wu and Wang, 2000), wheat (Gong *et al*., 2003) and cowpea (Anyia and Herzog, 2004).

Severe water stress may result in arrest of photosynthesis, disturbance of metabolism, and finally drying (Liang *et al*., 2006).

Chlorophyll 'A' and 'B' and total chlorophyll content were decreased in Cowpea and Alfa alfa under drought stress treatments with or without application of Glycine Betaine, but in applications of Glycine Betaine individually Chlorophyll 'A', 'B' and total Chlorophyll were increased significantly in both 100 ppm & 200 ppm of Glycine Betaine treatments in both the plants. The Chlorophyll content of the mature leaves of tomato, which were unfolded at a high temperature, was significantly high in the Crud Extract CE-applied plants compared to the Pure Extract PE-applied and control plants, reported by (Kanechi et al., 2012).

A reduction in chlorophyll content was reported in drought stressed soybean plants (Zhang *et al*., 2006). The chlorophyll content in the wheat leaf decreased due to chemical desiccation treatments (Shao *et al.,* 2006). The chlorophyll content decreased to a significant level at higher water deficits in maize and wheat plants (Nayyar and Gupta, 2006), *Vaccinium myrtillus* (Tahkokorpi *et al*., 2007) and in *Lysimachia minoricensis* (Galmes *et al*., 2007). A reduction in chlorophyll content was reported in drought stressed *Helianthus annuus* (Manivannan *et al*., 2007), Wheat (Lin and Wang 2002 and Gong *et al*., 2005), *Pinus halepensis* (Alonso *et al*., 2001), rice (Widodo *et al*.,2003), Cherry (Mauro Centritto, 2005), *Vaccinium myrtilllus* (Tahkokorpi *et al.*, 2007), Soybean (Zhang *et al*., 2007) and cotton (Massacci *et al.,* 2008).

The Carotenoid content in Cowpea and Alfa alfa was increased with Glycine Betaine treatments both 100 ppm & 200 ppm. Drought stress decreased the carotenoid content in all treatments 40% & 60% of irrigation water; it was high decreased in Alfa alfa, and also in the treatments of combining the applying of Glycine Betaine with the drought stress. Glycine Betaine did not improve Chl. 'b' and carotenoids of Sorghium plants grown under the stress conditions, reported by (A.H. Ibrahim & H.S. Aldesuquy; 2002).

Reduced carotenoid content under drought was reported in sunflower (Gimenez *et al*., 1992), *Nicotiana tabacum* (Delgado *et al*., 1992), Prairie grasses (Heckathorn *et al*., 1997), rice (Widodo *et al*.,2003), Wheat (Sawhney and Singh,2002, Lin and Wang, 2002, Gong *et al*., 2005), Cherry (Mauro Centritto, 2005), Soybean (De Ronde *et al*., 2004, Zhang *et al*., 2007) and *Litchi chinensis* (Damour *et al*., 2008).

The phenols content of the leaves in Cowpea was reduced in the treatments of applying Glycine Betaine, but it is increased in drought stress treatments of 60% of water without and with 100 ppm Glycine Betaine. In Alfa alfa the application of Glycine Betaine 200 ppm was induced the phenol content in the plants even when it combine the application of Glycine Betaine 100 ppm & 200 ppm with the drought stress treatments of 40% & 60% Its increased the phenol levels in the leaves except the treatment of 100 ppm with 60% of the irrigation water the phenols level was decreased.

Proline content was decreased in the treatments of applying the Glycine Betaine and in the water regimes treatments and in their combination, in both plants Cowpea and Alfa alfa.

Increased proline accumulation was reported in water stressed wheat (Hamada, 2000), Bell pepper (Nath *et al*., 2005) and sorghum (Yadav *et al*., 2005). Increased proline in the stressed plants may be an adaptation to overcome the stress conditions. The similar results were observed in wheat (Zhu *et al*., 2005; Vendruscolo *et al*., 2007), soybean (Heerden and Kruger, 2002), sorghum (yadav *et al*., 2005), and wheat (Pandey, 1982). Proline accumulation in plants might be a scavenger and acting as an osmolyte. The reduced proline oxidase may be the reason for increasing proline accumulation. Proline accumulated under stressed conditions supplies energy for growth and survival and thereby helps the plant to tolerate stress (Jaleel *et al*., 2007) and bell pepper (Nath *et al*., 2005). Proline may act as a non-toxic osmotic solute preferentially located in the cytoplasm or as an enzyme protectant, stabilizing the structure of macromolecules and organelles. Accumulated proline may supply energy to increase salinity tolerance (Mishra and Gupta, 2006, Jaleel *et al*., 2007). Proline as an osmo protectant compound plays a major role in osomo-regulation and osmotolerance (Demir, 2000). However, its definite role in exerting stress resistance continues to be a debate (Demiral and Turkan, 2006). The development of root system increases the water uptake and maintains requisite osmotic pressure through higher proline levels in *Phoenix dactylifera* (Djibril *et al*., 2005). Rapid decrease in proline levels after stress release may be one factor in resumption of growth after stress which is also an important determinant of overall stress tolerance (Hayano-Kanashiro *et al*., 2009). Regulation of cell death has obvious importance for incompatible plant-pathogen interactions, but may also be relevant to drought as plants with delayed senescence were reported to have dramatically improved drought resistance (Rivero *et al*., 2007).

Glycine Betaine contents in Cowpea was increased significantly in all the treatments of applications of Glycine Betaine and in treatments of water stress and in the combination of Glycine Betaine applications within the waters stress treatments

In Alfa alfa the Glycine Betaine content was reduced when it’s applied exogenously as a foliar spray to the leaves. In the treatments of drought stress the Glycine Betaine levels were rise up in both 40% & 60% of irrigation water. And in the combining the exogenous applying for Glycine Betaine with the drought stress conditions the levels of Glycine Betaine were increased in all treatments of 100 ppm & 200 ppm within 40% & 60% of irrigation water, except the treatments of 60% irrigation with 200 ppm the Glycine Betaine content was slightly reduced.

The glycine betaine content increased under drought stress in barley (Nakamura *et al.*, 2001), sunflower (Manivannan *et al*., 2007) and in higher plants (Jun *et al*., 2000). Aliphatic quaternary ammonium compounds (QAS) such as glycine betaine, stachydrine, homostachydrine, trigonelline have been found to accumulate in a large number of plants exposed to salt and water stress. The accumulation of glycine betaine might serve as an intercellular osmoticum and it can be closely correlated with the elevation of osmotic pressure (Jaleel *et al*., 2007b). The glycine betaine content increased under drought stress in *Radix astragali* (Tan *et al*., 2006). Glycine betaine, an important quaternary ammonium compound, is considered to be one of the most predominant and effective osmoprotectants. It is well established that its exogenous application might have some advantages as it improves drought tolerance in plants (Mahmood *et al*., 2009). It has been also reported earlier that rate and timing of GB application significantly affects drought tolerance ability of sunflower (Iqbal *et al*., 2008 and 2009). The glycine betaine accumulation was lowered in all the varieties in the recovery period. The fastest reduction in glycine betaine content was observed in cultivar S6 and the slowest was in S_2.

Effects of GB on Morphology

It has been reported that plants are able to utilize foliar-applied GB and to translocate it to almost all plant parts, especially developing organs (Makela *et al.*, 1996). Variation of their levels among different organs and at different ages were examined and GB content was markedly different among cotyledons, between roots, stems, leaves, and flowers (including seeds) (Wang *et al.,* 2004). The GB contents of these organs were very low during the earlier stages of plant development and increased as the plant developed. Roots accumulated a small amount of GB at all stage of plant development. The GB content was higher in leaves just before and after they unfolded and was lower in mature and older leaves. Salt stress triggered a marked induction of the production of GB in mature and old leaves (Wang *et al.,* 2004). Flowers (without seeds) contained GB, while the developing seeds had lower GB contents. It is suggested that the GB remains in dried seeds after it is imported from flowers and plants (Wang et al., 2004). Foliar applied GB is readily taken up and translocated within hours to developing leaves, probably along with assimilation products (Mackela *et al.,* 1996). However, the rate of uptake and consequent GB concentration in the plant tissue seem to be not only dependent on plant organ and its age but also on crop species and environmental factors (Mackela *et al.,* 1996). Coapplication of surfactant and vegetable oils in particular, tends to increase the GB uptake probably due to faster uptake rate and minimizing the risk of rain washing off of GB from the leaf foliage.

In addition, the stimulating effect of GB on plant growth may be attributed to an increase in the viability and uptake of water and essential nutrients through adjusting osmotic pressure in plant cells and by stabilizing many function units, like

oxygen evolving PSII complex, and ATP synthesis, membrane integrity, and enzyr activity (Tao and Gao, 2003). GB treatment fully overcame the adverse effects on CO_2 absorption and chlorophyll fluorescence during water stress (Weibin *et al.,* 1999).

Effects of GB on Proline

Proline accumulation is a common metabolic response of higher plants to water deficits, and salinity stress has been reviewed by numerous studies (Stewart and Larher, 1980; Thompson, 1980; Stewart, 1981; Hanson and Hitz, 1982; Delauney and Verma, 1993; Taylor, 1996).

This highly water soluble imino acid is accumulated by leaves of many halophytic higher plant species grown in saline environments (Stewart and Lee, 1974, in leaf tissues and shoot apical meristems of plants experiencing water stress, in desiccating pollen , in root apical regions growing at low water potentials and in suspension cultured plant cells adapted to water stress (Tal and Katz, 1980; Handa et al, 1986; Rhodes et al, 1986), Proline may also function as a protein-compatible hydrotrope (Srinivas and Balasubramanian, 1995), and as a hydroxyl radical scavenger (Smirnoff and Cumbes, 1989). The proline accumulated in response to water stress or salinity stress in plants is primarily localized in the cytosol (Leigh et al, 1981; Ketchum et al, 1991; Pahlich et al, 1983).

Effects of GB on Phenol content

Phenolic compounds also fulfil multiple roles in plants, as structural components of cell walls, participating in the regulation of growth and developmental processes, as well as in the mechanisms of defense against herbivores and pathogens; in addition, they are involved in the responses of plants to practically all types of abiotic stress. The increase in antioxidant phenolic compounds levels can be considered as part of the response induced to manage with oxidative stress.

Chapter 6: Conclusion

The results of the experiment show that the GB has increased the shoot height under drought stress of 60% of irrigation water, and it’s enhanced the root growth within the stress of 40% of irrigation water, GB also increased the stem diameter within the drought of 60% and increased the number of pods in treatment of drought stress 60% and application of 100 ppm of GB, Pigments Chlorophyll ‘a’, ‘b’, total Chlorophyll and Carotenoid increased within the application of GB on the not stressed plants. Application of GB it’s enhance the GB accumulation level in the plant and increased it under drought stress.

GB applications increased the Heavy metals levels in plants like Ca, Cu, Fe, K, Mg, Mn and S. From the results obtained from the experiments it can conclude that the GB has significant effects to ameliorate the drought stress on the Cowpea.

In the Alfa alfa the results collected in the experiment of applying exogenous GB within the stress of the drought indicate that, the GB has enhance the shoot growth and also increased it under the stress of drought within 60% of irrigation water. But there was no effect on the root length under the stress. Also GB applications have a significant effect in increasing the dry weight of the shoots and roots of the plants in both concentrations 100 & 200 ppm within the stress of irrigation water 60% & 40%.

There was no effect of GB on the Pigments Chlorophyll ‘A’, ‘B’, total Chlorophyll and Carotenoid within the drought stress on the plants. GB has a significant effect on raising up the phenols levels in Alfa alfa within the drought stress. Exogenous applications of GB were enhance the GB accumulation level in the plants within the drought stress of 40% of irrigation water. GB has no effects on heavy me

levels in the plants like: Ca, Cu, Fe, K, Mg and Mn, but it increased the Sodium and Sulfur content in plants significantly. .

As a conclusion of the effect of GB on the Alfa alfa growth and production under the drought stress, GB it’s enhanced the plants performance under the drought stress in both concentrations 100 & 200 ppm. In due of comparison of Cowpea and Alfa alfa it's found from this study that the GB has better effect on the Cowpea under drought stress than Alfa alfa.

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